Supporting information for

Dry heat as a decontamination method for N95 respirator reuse

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Text S1. Testing virus preparation

Tulane virus was received from Cincinnati Children's Hospital Medical Center[1] and rotavirus OSU strain was obtained from ATCC (VR-892). The MA104 cell line was used to propagate Tulane virus and rotavirus. The culture medium for the MA104 cells was prepared by mixing 1X minimum essential medium (MEM; Thermo Fisher Scientific, MA, USA) with 10% fetal bovine serum (FBS; Thermo Fisher Scientific, MA, USA), 1X antibiotic-antimycotic (Thermo Fisher Scientific, MA, USA), 17 mM of $NaHCO_3$, 10 mM of HEPES, and 1 mM of sodium pyruvate. MA 104 cells with 80-90% confluency were washed with PBS and inoculated with Tulane virus or rotavirus (OSU strain) in 175 cm^2 flasks at a multiplicity of infection (MOI) of 0.1. The inoculated cells were incubated at 37°C in a 5% CO_2 environment for an hour with gentle shaking every 10 to 15 min. Then, 20 mL of the culture medium were added to each flask. For Tulane virus, FBS was added to the culture medium at a final concentration of 2%. For rotavirus (OSU strain), trypsin was added to the culture medium at a final concentration of 10 µg/mL while FBS was not added. The infected flasks were incubated until an 80% cytopathic effect (CPE) was reached. The viruses were harvested after three freeze-thaw cycles. Both viruses were purified in 1 mM NaCl and 0.1 mM $CaCl_2$ solution using an ultracentrifuge (Optima XPN-90 Ultracentrifuge, Beckman Coulter, CA, USA). The ultracentrifuge was run at 1000 rpm (116 g) at 4°C for 5 min followed by 36000 rpm (150700 g) at 4°C for 3 hours. The final concentrations of Tulane virus and rotavirus were about 10^7 and 10^8 PFU/mL, respectively. The decontamination efficacy of both viruses was determined by plaque assay using the MA104 cell line. The incubation time for the plaque assay was 2 and 3 days for Tulane virus and rotavirus, respectively. Detailed information is described in our previous work.[2,3]

Adenovirus was obtained from ATCC (VR-846). They were propagated in A549 cells using Ham F-12 media with 10% FBS (Thermo Fisher Scientific, MA, USA) and 1X antibiotic-antimycotic (Thermo Fisher Scientific, MA, USA). The adenovirus was purified in 1X PBS (Thermo Fisher Scientific, MA, USA) using the ultracentrifuge and had a final infectivity of about 10^6 PFU/mL. A volume of 2 mL of overlay solution for the plaque assay was prepared by mixing 1.31 mL of 2X MEM, 0.5 mL of 1% agarose solution, 0.1 mL of FBS, 0.05 mL of 15 mM HEPES, 0.03 mL of 7.5% sodium bicarbonate, and 0.01 mL of 100X antibiotic-antimycotic. The incubation time for the plaque assay was 5 days. Detailed information is described in our previous work.[4]

Transmissible Gastroenteritis Virus (TGEV) was obtained from the Veterinary Diagnostic Laboratory at the University of Illinois at Urbana-Champaign. Although TGEV recognizes different receptors and infects different hosts from SARS-CoV-2, TGEV was chosen for this study because TGEV has a similar structure and the same genome type as SARS-CoV-2 (enveloped and (+)ssRNA). Both TGEV and SARS-CoV-2 belong to the *Coronaviridae* family and *Nidovirales* order. Both viruses have a single-stranded, positive-sense genomic RNA ranging from 28 to 30 kb. The genome is encapsulated by N protein forming nucleocapsid in common. Also, S protein, E protein, and M protein form spike protein, viral envelope, and membrane protein, respectively. Swine testis (ST) cells were used as a host cell for the virus to grow in and for the plaque assay. The same culture medium described for Tulane virus was also used for the ST cells. TGEV was harvested in the culture medium by centrifugation at 2000 rpm (556 g) for 10 min (Sorvall Legend RT Plus, Thermo Fisher Scientific, MA, USA), followed by filtration through a 0.45 μm filter (Millipore Sigma, MA, USA). The infectivity of TGEV was determined by the plaque assay; ST cell monolayers were prepared in 6-well plates (USA Scientific, FL, USA). The 750 μL of virus solution was inoculated to the cells followed by incubation at 37°C with 5% CO_2 for 60 min. The

virus solution was replaced with 2 mL of the MEM containing 1% agarose, 7.5% sodium bicarbonate, 15 mM HEPES, and 1X antibiotic-antimycotic. The overlay was solidified at 4°C for 20 min followed by the incubation at 37°C with 5% CO_2 for 4 days. The cellular monolayers were fixed with 10% formaldehyde for 1 hour. The plaques were visualized after the fixed cells were dyed with 0.05% crystal violet in 10% ethanol for 20 min. The initial infectivity of TGEV solution was about 10^6 PFU/mL.

The virus suspension was mixed with artificial saliva at a 1:1 ratio before use. The artificial saliva was prepared following ASTM E2720-16 with a slight modification.[5] In brief, 0.13 g of $CaCl_2 \cdot H_2O$, 0.42 g of $NaHCO_3$, 0.11 g of NH_4Cl, 0.88 g of NaCl, 1.04 g of KCl, and 3.00 g of porcine mucin was dissolved in 1000 mL distilled water.

Text S2. Experimental procedures for decontamination test

We followed three different procedures to test (1) the effect of the inoculation site on inactivation efficacy using Tulane virus, (2) the effect of the heat transfer method on inactivation efficacy using Tulane virus, and (3) the inactivation efficacy of dry heat over treatment time for each surrogate virus. (1) We inoculated each respirator with five separate 30 μL droplets of the Tulane virus and saliva mixture in five different locations: the inside edge, inside center, the outside edge, outside center, and the strap. The respirator was left in a biosafety cabinet until the testing solution had thoroughly evaporated (about 2 hours). We placed the contaminated respirator in the center of the electric cooker on top of paper towels so that the respirator was 3 cm above the bottom surface of the pot. These paper towels prevented direct contact between the respirator and the pot's hot surface. The respirator was subject to one 50-min cycle of 100°C dry heat. We then cut the treated respirator into 5 mm diameter pieces and submerged each in 1 mL of fresh culture medium. (2) We cut a clean respirator into 5 mm diameter pieces. We inoculated these pieces with 30 μL droplets of Tulane virus and saliva mixture, left the droplets to evaporate, and then wrapped the inoculated respirator pieces in a paper towel. We lined the interior of the pot with layers of polycotton fabric, placed the paper-towel-wrapped inoculated pieces in the center of the pot, and then covered the pieces with another layer of polycotton. The polycotton lining simulates respirators being stacked or enclosed in a bag so the dominant heat transfer method is convective heat instead of radiation heat from the interior walls of the pots. After the dry heat application, we added each piece to 1 mL of fresh culture medium. (3) For each of the four viruses (Tulane virus, rotavirus, adenovirus, and TGEV), we inoculated 5 mm diameter pieces of a clean respirator with 30 μL droplets of the virus and saliva mixture. After being left to evaporate in the biosafety cabinet, the inoculated respirator pieces were placed on paper towels in the electric cooker and subjected

to one 50-min cycle of 100°C dry heat. We then submerged each piece in 1 mL of fresh culture medium.

We detached the viruses from the respirator fragments by vortexing them in the culture medium for 3 min and shaking them for 30 min at 450 rpm (Figure S1). We followed the same procedure for the negative controls except that they were left in the biosafety cabinet instead of the electric cooker for the same amount of time as the dry heat treatment. The supernatant was used for the plaque assay and the molecular assays to determine the inactivation efficacy and mechanisms, respectively. We used the three molecular assays with a slight modification to analyze the primary structural target of Tulane virus by the dry heat treatment.[2,3] An RNase assay, a binding assay, and a two-step RT-qPCR assay were developed to examine the integrity of capsid proteins, binding proteins, and viral genomes, respectively (Text S3). We calculated the reduction in capsid protein integrity, binding protein integrity, intact RNA genome, and the virus infectivity by dividing the concentration of the negative control by that of the treated sample (i.e., $\log_{10}(N_0/N)$).

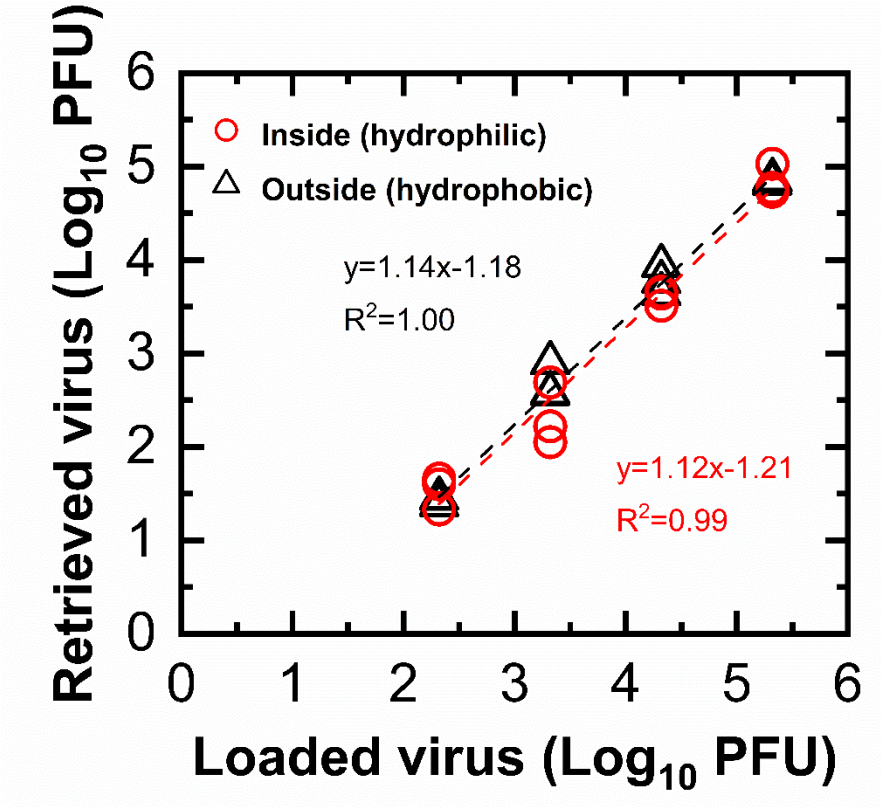

Figure S1. Calibration curve for virus detachment by 3 min vortex and 30 min shaking at 450 rpm. The detachment efficiencies were calculated by dividing the loaded virus from retrieved virus. The detachment efficiencies were not significantly different from inside and outside of the respirator pieces ($p>0.05$).

Text S3. Molecular assays to determine the primary damage of Tulane virus

We checked the impact of inhibition of viral RNA extract on RT-qPCR. The genomic RNA was extracted from the most concentrated TV sample (10^7 PFU/mL), serially diluted by 10 folds for RT-qPCR. The ΔCt values between the two consecutive dilutions were in the range from -3.3 to -3.6. The relationship between the Ct values and dilution factors presented linear relationship (R^2>0.99) in the tested range. Thus, we concluded inhibitions did not significantly affect RT-qPCR.[6]

The two-step RT-qPCR assay was designed to quantify intact genomic RNA of Tulane virus. This assay consisted of RT-PCR which synthesized cDNA covering 80% of the genomic RNA and qPCR which quantified the cDNA. We hypothesize that the viruses that had intact genomes in the range of the template for the cDNA will be quantified by this assay. The RNA was extracted from the viruses using QIAmp Viral RNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. The extracted RNA was mixed with ProtoScript First Strand cDNA Synthesis Kit (New England BioLabs, USA) following the manufacturer's protocol (i.e., 3 µL of RNA, 10 µL of M-MuLV Reaction Mix, 2 µL of M-MuLV Enzyme Mix, 2 µL of 10 µM of TV-VP2-R primer, and 3 µL of nuclease-free water). The reverse primer was designed to cover 5534 bp of genomic RNA. The cDNA was synthesized by the thermal cycler (MyCycler™, Bio-Rad). Finally, the cDNA was mixed with PowerUp SYBR™ Green Master Mix (Applied Biosystems, USA) (i.e., 2 µL of cDNA, 5 µL of Master mix, 0.3 µL of TV-NSP1-qPCR-F primer, 0.3 µL of TV-NSP1-qPCR-R primer, and 2.4 µL of nuclease-free water) followed by the qPCR (QuantStudio 3, Thermo Fisher Scientific). The qPCR efficiency of above 90% was determined based on calibration curves obtained by serially diluted solutions of synthetic cDNA. The sequence of this synthetic cDNA obtained from IDT is presented in Table S1. We also obtained another

type of calibration curve between TV infectivity and the corresponding genome copy of TV solutions (10^2 to 10^7 PFU/mL). This calibration curve showed a linear relationship (R^2=0.98) between infectivity and the number of genome copies (slope was 1.11). The molecular assays were then conducted within this linear range.

The RNase assay was developed to examine the integrity of capsid proteins. The RNase (A/T1 mix, Thermo Fisher Scientific, USA) was incubated with the viruses at 37°C for 30 min. We assumed that the RNase would be able to penetrate the damaged-capsid and degrade the RNA if the capsid proteins were damaged. RNase inhibitor (SupeRNase inhibitor, Sigma Aldrich, USA) reacted with the RNase treated solution at room temperature for 30 min to inhibit the RNase activity. The remaining intact RNA was quantified by two-step RT-qPCR, which represented the integrity of capsid proteins.

The binding assay measures the integrity of binding proteins. Magnetic beads (MagnaBind carboxyl-derivatized beads, Thermo Fisher Scientific, USA) loaded by porcine gastric mucin (Sigma Aldrich, USA) were mixed with the virus solution. The viruses with intact binding proteins were bound to the magnetic beads while the viruses that lost binding ability were washed out. The viruses bound to the magnetic beads were quantified by one-step RT-qPCR (QuantStudio 3, Thermo Fisher Scientific) with iTaq universal SYBR green reaction mix (Bio-Rad Laboratories, USA) following the manufacturer's protocol. Each reaction included 3 μL of RNA, 5 μL of 2 × iTaq universal SYBR green reaction mix, 0.125 μL of iScript reverse transcriptase, 0.3 μL of 10 μM TV-NSP1-qPCR-F primer, 0.3 μL of 10 μM TV-NSP1-qPCR-R primer, and 1.275 μL of nuclease-free water. We also obtained a calibration curve for the binding assay with serially diluted TV ranging from 10^3 to 10^7 PFU/mL. A linear relationship between viral infectivity and genome copies after the binding assay (R^2=1.00 and a slope of 0.95) suggested that the binding assay

provide a reliable outcome in the range of 10^3 to 10^7 PFU/mL. The qPCR efficiency was greater than 85%. Detailed information for the two-step RT-qPCR, RNase, binding assay are described in our previous work.[2,3]

Table S1. Information about RT-qPCR conditions and primers

Assay name	process	Primer name	Sequence (5'-3')	Position in the genome	Amplicon length (bp)	Reaction conditions
Two-step RT-qPCR	cDNA synthesis	TV-VP2-R	AGCGAGAGAAAAGCCTGCA	6213-6232	5354	42°C for 60 min and 80°C for 5 min
	qPCR	TV-NSP1-qPCR-F	GTGCGCATCCTTGAGACAAT	879-899	132[1)]	95°C for 10 min, 40 cycles of (95°C for 15 s, 60°C for 1 min)
		TV-NSP1-qPCR-R	TTGGAGCCGGGTAGAAACAT	991-1011		
One-step RT-qPCR (binding assay)	RT-qPCR	TV-NSP1-qPCR-F	GTGCGCATCCTTGAGACAAT	879-899	132[1)]	50°C for 10 min and 95°C for 1 min, followed by 40 cycles of (95°C for 10 s, 60°C for 30 sec)
		TV-NSP1-qPCR-R	TTGGAGCCGGGTAGAAACAT	991-1011		

1) The sequence of standard sample for the NSP gene of Tulane virus (Integrated DNA technologies, USA): 5'CCGTGGTTGTGCGCAGTATTGGAAACACAAACATTGCTGGGAAATTCCTCAACGTCTTCACAGGTACAGTTGTGGCAGCTGGGAAGAAATCTGACGGCCTCGGGTCTGAACCAGGAGACTGTGGCTCACCATATCTTAAATTTGTTAATGGAAAACCAACTCTTGTAGGCATTCACACAGCAGGCAGCTACACTACCAACCAGGTTGCAGGCTTAGTGATACCTTCTAGATTCAACCTTG-3' (GenBank accession number: EU391643).

Text S4. NaCl Particle Filtration Efficiency Test

A schematic of the particle filtration testing design used in this study is shown in Figure S2. We built a polypropylene chamber with various fittings and valves to control the aerosol concentration inside the chamber. The chamber inlet valve was connected to an aerosol generator (TSI Constant Output Atomizer Model 3076). The atomizer was filled with 2% NaCl solution (which is commonly used for measuring the penetration efficiency of N95 masks[7] in Milli-Q water to generate polydisperse particles (10-800 nm) at a relatively constant rate. The count median diameter of the droplets generated by the atomizer is expected to lie between 80 and 150 nm.[8] At the inner roof of the chamber, a small fan was installed to mix the air and thus minimize spatial heterogeneity of the particle concentration inside the chamber. A vent on the roof was also provided to connect it to the compressed air, which was used to dilute the concentration of the particles inside the chamber. The aerosols generated from the atomizer were first dried by passing it through a custom-built diffusion dryer (22 in. long and 3 in. diameter tube with a concentric meshed tube for airflow), filled with 2 mm – 4 mm silica gel. The dry aerosols were then passed through a custom-built aerosol neutralizer (1" diameter and 10" long stainless steel tube with 4 Staticmaster® 2U500, 3" Ionizing Cartridges glued inside it)[9] to neutralize excess charge on the aerosols' surface. A conductive tubing was passed through the chamber and connected to a particle counter (Condensation Particle Counter, CPC, TSI, Model 3022A; flow rate = 1.5 lpm) to measure the particle concentration. Thus, a steady-state concentration of the aerosols (~45,000 particles/cm^3) was maintained inside the chamber. A small circular section of the mask was loaded into a 47 mm filter holder (URG, Carrboro, NC, USA) and air was drawn at a specific flow rate, measured by an inline flow meter (4-50 slpm; Dwyer Instruments, MI, USA) using a vacuum line. The surface area of the N95 mask was measured manually (~150 cm^2) to calculate face velocity

for the NIOSH recommended flow rate (i.e. 85 lpm). The face velocity for this recommended flow rate is 9.4 cm/s. Since, we used only a small section (47 mm diameter) of this mask, we drew only 10 lpm through the filter holder, which yielded an equivalent face velocity of 9.4 cm/s. Out of the total flow through the filter, CPC used 1.5 lpm, while the rest was by-passed through a T-connector. A pressure gauge (Magnehelic 1-10 inches of water) was also connected in parallel, right downstream of the filter holder using a T-connector to measure the pressure drop. The particle number concentration was measured before and after connecting the filter holder, and particle removal efficiency of the mask was measured by the following equation:

$$Particle\ removal\ Efficiency\ (\%) = \left(1 - \frac{particle\ number\ concentration\ after\ placing\ the\ mask\ \left(\frac{\#}{cm^3}\right)}{particle\ number\ concentration\ before\ placing\ the\ mask\ \left(\frac{\#}{cm^3}\right)}\right) \times 100$$

Note, the NIOSH testing protocol recommends performing the filtration tests until the respirator reaches a loading of 200 mg NaCl (this takes around 90-100 min);[10] however, in our current study we stopped the testing once a constant particle filtration value was obtained (10 - 15 min of total sampling time). We assume this reduced sampling time would not significantly influence our results based on several past studies showing that the filtration efficiencies obtained by measuring initial penetration (average of the first min) of N95 masks were similar to the penetration levels obtained at full loading conditions (i.e. 200 mg).[10,11] NISOH recommends N95 masks should not exceed peak air flow resistance of 35 mm (1.37 inches of water). Here, in addition to the particle

filtration efficiency we also report the pressure drop across the filter after every cycle of rice cooker decontamination to observe any effect on the inhalation resistance.

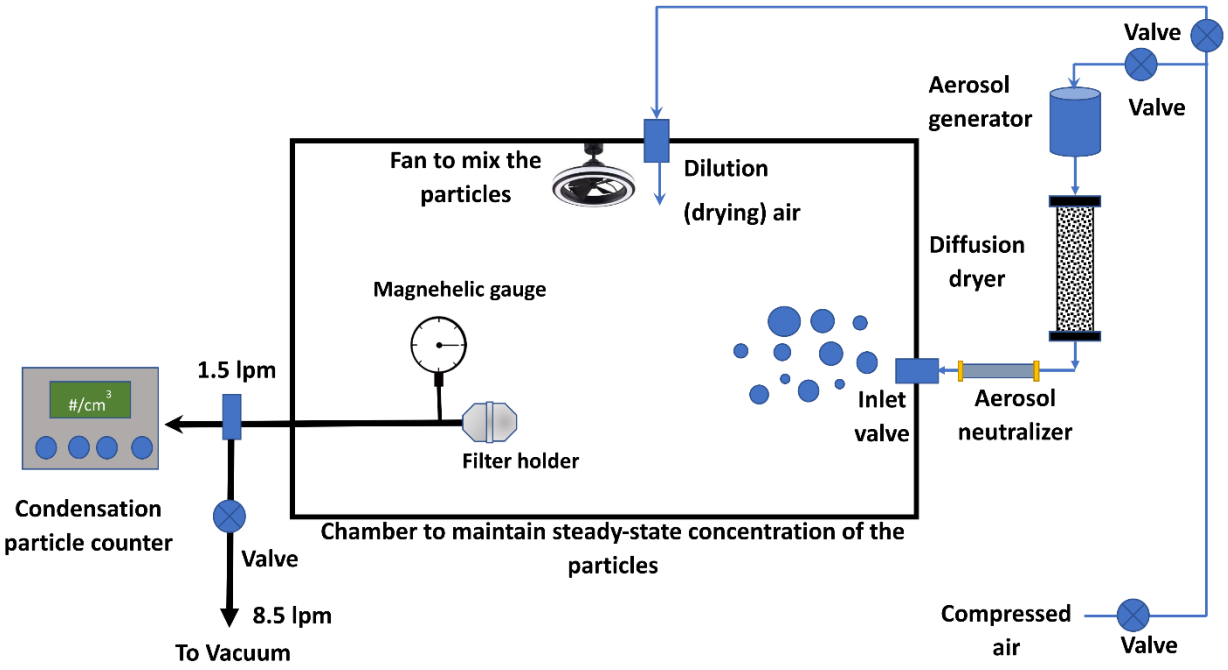

Figure S2. Experimental setup for testing the NaCl particle filtration efficiency of the respirator.

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